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FOOD MICROBIOLOGY

Proximity to Other Commercial Turkey Farms Affects Colonization Onset, Genotypes, and Antimicrobial Resistance Profiles of *Campylobacter* spp. in Turkeys: Suggestive Evidence from a Paired-Farm Model

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ABSTRACT *Campylobacter* is a leading foodborne pathogen, and poultry products are major vehicles for human disease. However, determinants impacting *Campylobacter* colonization in poultry remain poorly understood, especially with turkeys. Here, we used a paired-farm design to concurrently investigate *Campylobacter* colonization and strain types in two turkey breeds (Hybrid and Nicholas) at two farms in eastern North Carolina. One farm (the Teaching Animal Unit [TAU]) was a university teaching unit at least 40 km from commercial turkey farms, while the other (SIB) was a commercial farm in an area with a high density of turkey farms. Day-old birds were obtained from the same breeder flock and hatchery and placed at TAU and SIB on the same day. Birds were marked to identify turkey breed and then commingled on each farm. TAU birds became colonized 1 week later than SIB and had lower initial *Campylobacter* levels in the cecum. Interestingly, *Campylobacter* genotypes and antimicrobial resistance profiles differed markedly between the farms. Most TAU isolates were resistant only to tetracycline, whereas multidrug-resistant isolates predominated at SIB. Multilocus sequence typing revealed that no *Campylobacter* genotypes were shared between TAU and SIB. A bovine-associated genotype (sequence type 1068 [ST1068]) predominated in *Campylobacter coli* from TAU, while SIB isolates had genotypes commonly encountered in commercial turkey production in the region. One multidrug-resistant *Campylobacter jejuni* strain (ST1839) showed significant association with one of the two turkey breeds. The findings highlight the need to further characterize the impact of farm-specific factors and host genetics on antimicrobial resistance and genotypes of *C. jejuni* and *C. coli* that colonize turkeys.

IMPORTANCE Colonization of poultry with *Campylobacter* at the farm level is complex, poorly understood, and critically linked to contamination of poultry products, which is known to constitute a leading risk factor for human campylobacteriosis. Here, we investigated the use of a paired-farm design under standard production conditions and in the absence of experimental inoculations to assess potential impacts of farm and host genetics on prevalence, antimicrobial resistance and genotypes of *Campylobacter* in commercial turkeys of two different breeds. Data suggest impacts of farm proximity to other commercial turkey farms on the onset of colonization, genotypes, and antimicrobial resistance profiles of *Campylobacter* colonizing the birds. Furthermore, the significant association of a specific multidrug-resistant

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Campylobacter jejuni strain with turkeys of one breed suggests colonization partnerships at the *Campylobacter* strain-turkey breed level. The study design avoids potential pitfalls associated with experimental inoculations, providing novel insights into the dynamics of turkey colonization with *Campylobacter* in actual farm ecosystems.

KEYWORDS *Campylobacter*, antimicrobial resistance, colonization, genotype, turkey

Campylobacter is a leading zoonotic foodborne pathogen, with an estimated 0.8 million cases of human disease annually in the United States alone (1). Contaminated poultry products are considered a major vehicle for human campylobacteriosis (2–4). In the United States and other industrialized nations, *Campylobacter jejuni* is implicated in the majority (approximately 85%) of human campylobacteriosis cases, with *Campylobacter coli* accounting for most of the remaining cases (1, 5).

Factors that contribute to the colonization of poultry flocks with *Campylobacter* remain poorly understood and have been primarily investigated with broilers. In addition to impacts stemming from the environment and management practices in the poultry production ecosystem (6–13), several studies also point to the importance of host genetics in colonization of the birds with *Campylobacter* (14–20). Markedly less information is available for turkeys, in spite of the fact that turkey flocks are frequently colonized with *C. jejuni* and *C. coli*, including strains with multiple antimicrobial resistance attributes (8, 9, 21–28).

In an earlier study, we investigated *Campylobacter* colonization in two different turkey flocks that were grown concurrently and derived from the same breeder flock. One flock was associated with a university teaching unit, while the other was at a farm operated under the control of a commercial turkey company in the same region (29). It was found that birds on the industry-operated farm became colonized with *Campylobacter* spp., mostly *C. coli*, within 2 weeks and remained colonized until processing, while *Campylobacter* could not be detected in the ceca of the birds from the university farm. The findings suggested a pronounced impact of farm-related factors on *Campylobacter* colonization and failed to provide evidence for vertical transmission of *Campylobacter* in turkeys (29).

The objective of the current study was to further assess the usefulness of a paired-farm system as a model to analyze the dynamics of *Campylobacter* colonization in turkeys. In addition, we wished to determine the potential impact of host genetics in colonization by including birds of two different breeds extensively employed in commercial turkey production.

RESULTS AND DISCUSSION

The onset of *Campylobacter* colonization differed between the farms. The two farms were concurrently monitored for *Campylobacter* at weekly intervals over the entire production period, i.e., weeks 1 to 9. Both flocks became colonized with *Campylobacter*, but the timing and initial levels in the cecum were different. *Campylobacter* was detected 1 week later at TAU (week 3) than at SIB (week 2) (Table 1). During the first 2 weeks of colonization, levels of *Campylobacter* (CFU/g cecal content) were also lower at TAU than at SIB. However, by week 5, cecal levels were similar between the two farms and remained similar for the remainder of the study (Table 1). During the first 2 weeks of TAU colonization (weeks 3 and 4), *Campylobacter* levels were lower in turkeys of breed Hybrid, but levels were similar between the two breeds thereafter (Table 1). We were unable to detect a similar difference between the two breeds during the first weeks of colonization in SIB (Table 1). The data suggest that potential breed-associated differences in *Campylobacter* levels in the cecum are transient and may be best detectable when total levels are relatively low, as was the case in the first 2 weeks of colonization of TAU (Table 1).

***Campylobacter* antimicrobial resistance profiles and genotypes differed markedly between the two farms.** We analyzed a total of 110 *Campylobacter* isolates, including 48 from TAU and 62 from SIB, with an average of 4 isolates per positive cecal

TABLE 1 *Campylobacter* colonization at SIB and TAU

Week(s)	*Campylobacter* colonization (CFU/g cecal content) at:			
	SIB		TAU	
	Hybrid	Nicholas	Hybrid	Nicholas
1	ND[a]	ND	ND	ND
2	7.20×10^5	6.92×10^5	ND	ND
3	2.80×10^7	4.00×10^6	6.00×10^3	3.00×10^5
4	5.60×10^6	3.00×10^7	2.00×10^5	8.40×10^5
5–9	2.75×10^7	5.56×10^7	8.94×10^6	2.37×10^7

[a]ND, not detected.

sample (range, 1 to 6 isolates/positive sample). *C. coli* predominated at both farms, both in brooders (87.5 and 93.8% of the *Campylobacter* isolates in SIB and TAU, respectively) and in grow-out birds (83.9 and 93.8% in SIB and TAU, respectively). All remaining *Campylobacter* isolates were *C. jejuni*.

Even though the same *Campylobacter* species (*C. coli*) predominated at both farms, the antimicrobial resistance (AMR) profiles of the isolates differed significantly ($P < 0.0001$). TAU isolates were primarily *C. coli* resistant only to tetracycline (designated CC: T), while a variety of other AMR profiles were noted among isolates from SIB (Fig. 1). Interestingly, the few *C. jejuni* isolates encountered at TAU had the same AMR phenotype as the TAU *C. coli* isolates, i.e., resistance only to tetracycline (designated CJ: T). In contrast, all tested *C. jejuni* isolates from SIB were multidrug resistant, being susceptible only to erythromycin (AMR profile TSKQG) (Fig. 1). The only species/AMR profile combination encountered in both SIB and TAU was *C. coli* with resistance to tetracycline and the quinolones nalidixic acid and ciprofloxacin (AMR profile TQ) (Fig. 1). *C. coli* TQ was encountered just once at TAU (week 4), but from multiple time points at SIB (Fig. 1 and Table 2). However, as discussed below, the *C. coli* TQ isolates in TAU and SIB had unrelated genotypes.

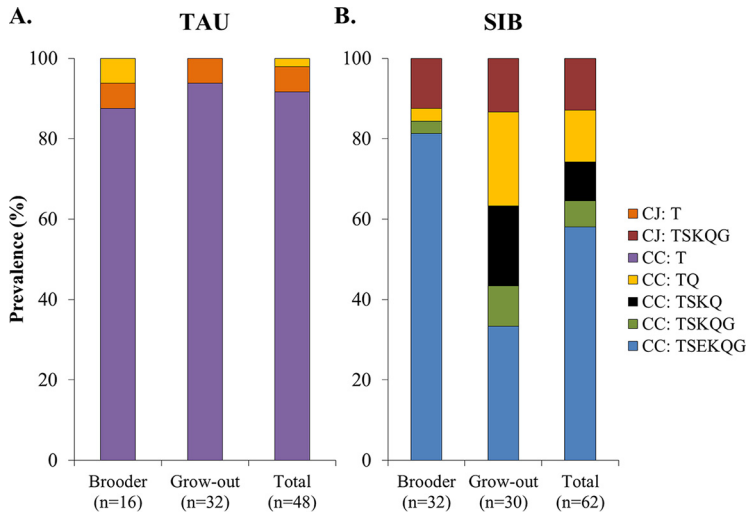

FIG 1 *Campylobacter* species and AMR profiles at the two farms. Isolates were from cecal contents of birds from (A) TAU and (B) SIB. Species and AMR profile determinations were as described in Materials and Methods. Findings are shown for brooders (from onset of colonization in week 2 or 3 to week 5), grow-out birds (weeks 6 to 9), and total duration of the study (from onset of colonization to week 9) for each farm. CJ and CC in the color code inset indicate *C. jejuni* and *C. coli*, respectively. AMR profile acronyms indicate resistance to tetracycline (T), streptomycin (S), kanamycin (K), erythromycin (E), the quinolones nalidixic acid and ciprofloxacin (Q), and gentamicin (G); combination of letters indicates specific AMR profile, e.g., T, resistance only to tetracycline; TQ, resistance to tetracycline and quinolones; TSEKQG, resistance to all tested antimicrobials listed above; TSKQG, resistance to all tested antimicrobials listed above except for erythromycin.

TABLE 2 AMR profiles and genotypes of *C. jejuni* and *C. coli* from TAU and SIB at different time points

Farm	Species	AMR profile[a]	Time (weeks)[b]	ST[c]
TAU	*C. coli*	T	**3**, 4, **5**, **6**, 7, **8**, **9**	1068
	C. coli	TQ	**4**	1068
	C. jejuni	T	**5**, **6**, 8	1698
SIB	*C. coli*	TSEKQG	**2**, **3**	1604
			5, **7**	1149
			8	**9064**
	C. coli	TQ	**5**	1161
			8, **9**	**8094**
	C. coli	TSKQ	**8**	889
			9	**8094**
	C. coli	TSKQG	5, 6, 7	ND[d]
	C. jejuni	TSKQG	2, **5**, **6**, **7**	1839

[a]Abbreviations are as indicated in the legend to Fig. 1.
[b]Boldface indicates weeks from which isolates were genotyped by MLST.
[c]Boldface indicates a novel ST.
[d]ND, not determined because isolates could not be resuscitated.

A total of 24 isolates, representing different species/AMR profile combinations at each farm, were analyzed by multilocus sequence typing (MLST). The findings revealed the absence of common *Campylobacter* strains shared between the two farms (Fig. 2). In fact, the sole AMR profile (*C. coli* TQ) shared between the two farms corresponded

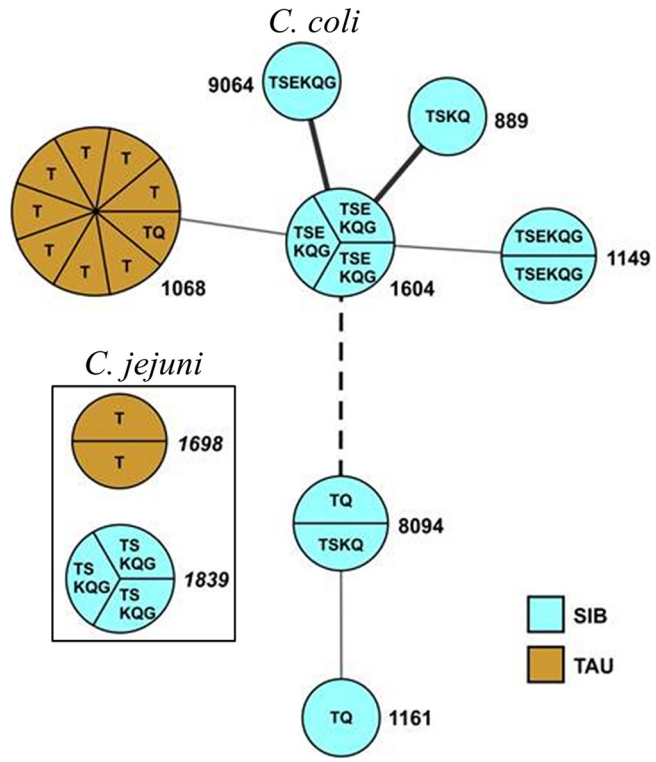

FIG 2 Minimum spanning tree of MLST-based STs in *Campylobacter* isolates from TAU and SIB. TAU and SIB are indicated in brown and blue, respectively. STs encountered in *C. jejuni* are italicized and separated from the rest of the STs within a black rectangle, while *C. coli* STs are in normal font. Thick solid line, 2-allele difference; thin solid line, 3-allele difference; dashed line, 5-allele difference. STs not linked with any lines (ST1698 and ST1839) shared no alleles with any of the other shown STs (i.e., 7-allele difference). Sizes of circles correspond to numbers of isolates with the corresponding ST, and circle segments corresponding to each isolate are indicated for STs encountered in more than one isolate. Letters inside the circles indicate the antimicrobial resistance profile, as described in the legend to Fig. 1.

to unrelated sequence types. *C. coli* TQ from TAU had sequence type 1068 (ST1068), which was clearly distinct from *C. coli* TQ isolates from SIB, which had either ST1161 or ST8094 (Fig. 2). ST1068 differed markedly from ST1161 (all seven alleles) and ST8094 (six alleles), indicating that *C. coli* TQ from TAU and SIB represent unrelated strains. *C. coli* T isolates which predominated at TAU and were obtained at multiple time points between weeks 3 to 9 had ST1068, which was also noted as mentioned above in the sole *C. coli* TQ strain from TAU (Table 2 and Fig. 2). ST1068 was not detected among any of the *C. coli* SIB isolates, which instead exhibited six different STs, two of which (ST8094 and ST9064) were novel STs found in this study (Table 2 and Fig. 2). None of the *C. coli* STs encountered in SIB were detected in TAU (Fig. 2).

Similarly to findings with *C. coli*, genotypes of *C. jejuni* were unrelated between TAU and SIB; that is, *C. jejuni* isolates from TAU had ST1698, while SIB isolates had ST1839, with the two STs differing at all 7 alleles (Fig. 2). An earlier analysis of isolates from commercial turkeys in the same region (eastern North Carolina) indicated that *C. jejuni* isolates of ST1698 were uncommon, being encountered only twice among 80 isolates, but exhibited the same AMR profile (resistance only to tetracycline) as the TAU isolates (22). In contrast, *C. jejuni* ST1839 has been frequently encountered in commercial turkey flocks in this region (22, 30).

SIB isolates exhibited notable strain compositional shifts between brooder and grow-out. No major strain composition shifts between brooders and grow-out birds were noted in TAU, where *C. coli* T was predominant in brooders (14/16, 87.5%) and remained predominant in grow-out birds (30/32, 93.8%) (Fig. 1). However, a significant difference ($P = 0.0004$) in strain distribution between total brooder and grow-out bird isolates was observed. This difference was most apparent in SIB, where four strain profiles detected in brooders were also detected in grow-out birds, but at different proportions (Fig. 1). In addition, *C. coli* isolates with a new multidrug resistance profile (resistance to tetracycline, streptomycin, kanamycin, and the quinolones ciprofloxacin and nalidixic acid, i.e., AMR profile TSKQ) were noted only in grow-out birds (Fig. 1). Especially noteworthy was the shift in the incidence of *C. coli* resistant to all tested antibiotics, i.e., AMR profile TSEKQG. These isolates predominated in brooders (26/32, 81.3%), but were less common (10/30, 33.3%) among grow-out isolates (Fig. 1). Their prevalence decreased as birds aged, being encountered among 9 of the 16 total *Campylobacter* isolates (56.3%) from weeks 6 and 7, only once among 6 isolates at week 8, and not at all among the 8 isolates at week 9.

Competition by new strains colonizing the older birds, such as *C. coli* TSKQ, may have contributed to the observed decreases in *C. coli* TSEKQG as birds aged. In addition, fitness of different *Campylobacter* strains may change in response to age-related changes in the cecal microbiota (31), and this may result in displacement of previously common strains such as *C. coli* TSEKQG. The potential impacts of gut microbial community shifts on *Campylobacter* strain dynamics remain to be elucidated.

Evidence for strain-dependent impact of host genetics. Strain (*Campylobacter* species/AMR profile) distributions among all isolates were significantly different between breeds ($P = 0.0059$). The most apparent difference was observed with *C. jejuni* TSKQG (ST1839). This strain was detected in 8/52 (15%) of the total Nicholas isolates but was not detected among any of the 58 isolates from Hybrid birds ($P = 0.0018$). More specifically, this strain accounted for 4/16 (25%) and 4/14 (28.6%) of isolates from Nicholas brooders and grow-out birds, respectively, i.e., 26.7% of the total Nicholas-derived isolates from SIB, but it was not detected among any of the 32 isolates from Hybrid birds at the same farm (SIB) ($P = 0.0017$). *C. jejuni* TSKQG (ST1839 and related STs) is a widely disseminated *C. jejuni* clone in turkey farms in eastern North Carolina (22, 30), possibly reflecting high colonization fitness in

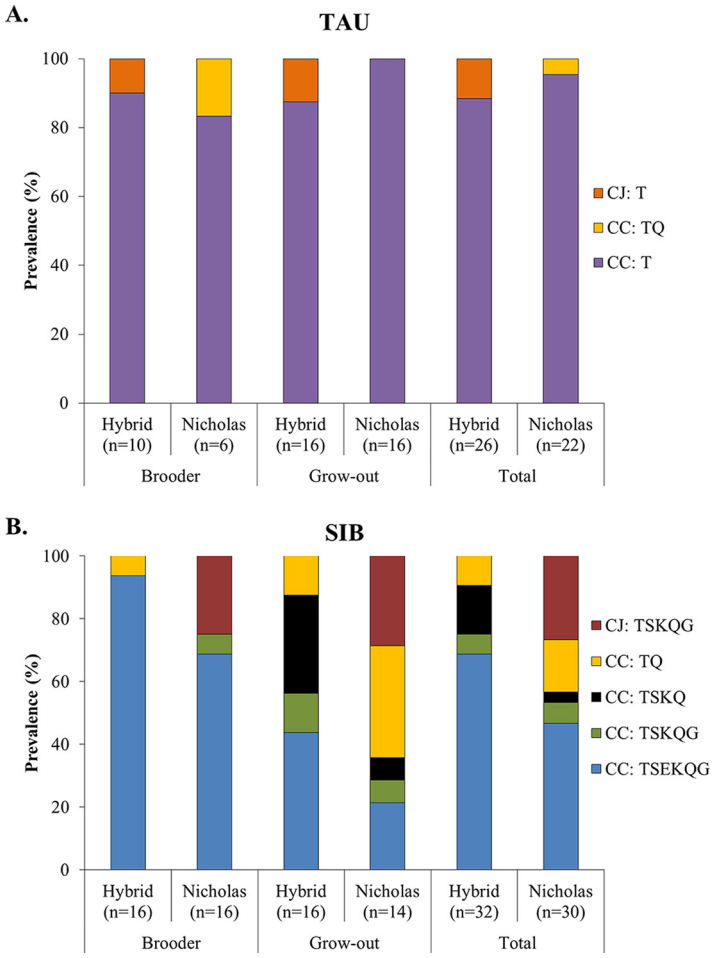

FIG 3 *Campylobacter* species and AMR profiles in turkeys of breeds Hybrid and Nicholas at (A) TAU and (B) SIB. Species and AMR determinations of isolates obtained from cecal contents were as described in Materials and Methods. Findings are shown for brooders (from onset of colonization in week 2 or 3 to week 5), grow-out birds (weeks 6 to 9), and total duration of the study (from onset of colonization to week 9) for each breed. Species and AMR profile acronyms in the color code inset are as in the legend to Fig. 1.

certain turkey breeds. Certain other strains from SIB, e.g., *C. coli* with AMR profile TSEKQG, also showed breed-associated differences (Fig. 3).

TAU birds of either breed were predominantly colonized by *C. coli* resistant only to tetracycline (Fig. 3). As discussed above, *C. jejuni* was uncommon in TAU, but when encountered, it was ST1698 and resistant only to tetracycline. It is worthy of note, however, that *C. jejuni* T isolates from TAU were only detected in Hybrid birds (Fig. 3).

Even though substantial evidence indicates the impact of host genetics for *Campylobacter* colonization of chickens (14–20), relevant reports have been lacking for turkeys. In addition, previous analyses typically employed experimental infections of chickens with a single *Campylobacter* strain and thus could not assess preference of different *Campylobacter* strains for different breeds of poultry. However, a study that tested a panel of *C. jejuni* strains with two chicken breeds identified potential breed- and strain-dependent differences in extraintestinal spread of *C. jejuni* to the liver (19). In chickens, resistance to *C. jejuni* colonization is a complex trait accompanied by multiple immunomodulatory responses (17, 18, 20, 32, 33), and it is reasonable to expect similar complexity in the case of turkeys. Future studies may unravel mechanisms underlying colonization dynamics at the strain level for both *Campylobacter* and its avian host.

***Campylobacter* strain types and AMR profiles may reflect proximity to other farms.** The finding that most isolates from TAU birds were *C. coli* with ST1068 was surprising, as this ST was previously detected frequently among *C. coli* from cattle and,

to a lesser extent, from swine but not from poultry (34, 35). Even though *Campylobacter* strains may be able to transfer between poultry and cattle (13), the source of the *C. coli* ST1068 isolates predominating at TAU is currently unknown. On the other hand, all six *C. coli* STs detected in SIB were either identical or related to STs such as ST889 and ST1161 (Fig. 2) that were previously frequently detected among *C. coli* isolates from commercial turkey farms (34, 36). Such STs were uncommonly encountered among *C. coli* isolates from other animal hosts (34, 35), suggesting that they represent turkey-adapted strains.

Similar findings were obtained for *C. jejuni* strains. ST1698, detected at TAU, was only rarely detected before among *C. jejuni* isolates from commercial turkey farms in North Carolina (22, 30). In contrast, ST1839, which was detected in SIB, appears to belong to a turkey-associated clone frequently encountered among *C. jejuni* isolates from commercial turkeys in North Carolina (22, 30) (W. G. Miller and S. Kathariou, unpublished findings).

Such findings may reflect a common pool of *Campylobacter* strains shared among farms in a region dense in turkey farms, such as where SIB was located. Even though cattle and swine are also frequently produced in this turkey-dense region (23, 37) (H. Bolinger and S. Kathariou, unpublished), there is evidence for host preference among *Campylobacter* strains from different agricultural animal hosts, especially for *C. coli* (34, 35). Transmission of *Campylobacter* from one turkey farm to another may be facilitated by human and equipment traffic, flies, and other vectors (21). In addition, commercial farms in the vertically integrated turkey industry typically employ the same service staff to visit multiple farms, providing opportunities for the spread of *Campylobacter* strains from farm to farm (H. Bolinger, D. Carver, and S. Kathariou, unpublished).

One can speculate that in the absence of nearby commercial turkey farms, as was the case for TAU, birds can still become colonized by strains from other *Campylobacter* reservoirs in the vicinity. In the current study, the sources and transmission routes of *Campylobacter* into the TAU turkey farm remain unidentified. Nonetheless, the data suggest that, even though *C. coli* ST1068 has not before been encountered in commercial poultry and appears to be associated primarily with mammalian sources, especially cattle, it can still colonize turkeys. Under natural conditions, such colonization capacity can be best demonstrated when the birds are at a distance from other commercial turkey flocks, as was the case here, and thus do not have easy access to the *C. jejuni* or *C. coli* strains commonly associated with commercial turkey production.

Conclusions. This paired-farm study indicated a lag in colonization at one farm (TAU) in comparison to the other (SIB). Even though both farms were in the same geographical region (eastern North Carolina), only SIB was in an area with a high density of commercial turkey farms; TAU was at a distance of $>$40 km from commercial turkey farms. The complete lack of overlap in *Campylobacter* genotypes between the two farms was of special interest. It was also noteworthy that multidrug-resistant strains predominated in SIB, whereas virtually all *Campylobacter* isolates from TAU were resistant only to tetracycline.

Differences in colonization onset and in *Campylobacter* strains between the two farms likely reflect differences in access and sources of *Campylobacter*. This was supported by the finding that SIB strains had genotypes commonly found in commercial turkey production from the region, in contrast to TAU birds, which were colonized by *Campylobacte*r strains with genotypes that are absent or rarely encountered in commercial turkey flocks in the region.

The importance of farm location to the types of *Campylobacter* strains colonizing the birds could not be ascertained from a previous paired-study longitudinal analysis in the same region, where the birds at one of the farms (in the same facility as the current TAU) remained free of *Campylobacter* for the entire study, while birds at the other site became colonized (29). The current study design, furthermore, allowed us to assess potential impacts of turkey breed on the *Campylobacter* status of the birds at each farm. Even though birds of both tested breeds became colonized with *Campylobacter* at both

farms, it was noteworthy that a specific *C. jejuni* strain encountered at the SIB farm showed significant association with one of the two turkey breeds. The findings suggest the potential for preferred *Campylobacter* genotype-turkey breed partnerships and warrant further studies.

Even though SIB was operated under conditions standard to the industry and was located in a turkey-dense region, analysis of additional farms from this and other regions would be valuable. It will be of special interest to determine whether the breed preference observed here can be observed in other turkey farms colonized with the same strains and whether *Campylobacter* strains predominating in other regions may also show preference for certain turkey breeds. The current study was ideally designed to assess the impact of turkey breed in a commercial setting, as birds of each breed were physically marked and then commingled on the same farm and at the same time. This type of study is logistically not easy to do, especially in a commercial setting. Even though experimental inoculations of turkeys with mixtures of specific strains can also be employed to assess potential breed-associated competitive advantages of specific strains, further investigations of flocks which are naturally colonized with *Campylobacter* during production will be especially useful, as they better reflect the complexities of the poultry production ecosystem.

In conclusion, the paired-farm study described here has provided considerable insight into the dynamics of *Campylobacter* colonization in turkeys, while also clearly illustrating the complexity of *Campylobacter* transmission and adaptations in poultry production. Further studies are needed to elucidate transmission of *Campylobacter* during agricultural production of turkeys and other poultry, leading to improved intervention strategies.

MATERIALS AND METHODS

Paired-farm design and flock management. Birds were of two commercial turkey breeds, Hybrid and Nicholas, and originated from a single hatchery and breeder source. Day-old poults were randomly assigned and transferred on the same day to either the Teaching Animal Unit (TAU) farm, also employed in our previous study (29), at North Carolina State University, College of Veterinary Medicine (Raleigh, NC), or a commercial farm (sibling production, designated SIB) in a rural area with a high density of turkey farms. Both TAU and SIB were in eastern North Carolina. Turkey breeds Hybrid and Nicholas were physically marked (clipping of a claw on the left versus right leg) and then commingled and raised as a single flock at each farm. Stocking densities for SIB and TAU were 21.5 and 30.1 birds/m^2, respectively. Totals of 2,000 birds and 8,000 birds were placed at TAU and SIB, respectively. SIB birds were in one house during the brooder stage (weeks 1 to 5) but were moved at 5 weeks to a different house at the same farm for the grow-out stage (weeks 6 to 9), while TAU birds remained in the same turkey house during the entire 9-week trial. Feed was the same for both TAU and SIB and included nitarsone at 170.1 g/ton to help prevent blackhead, caused by *Histomonas meleagridis*. General management and biosecurity practices followed industry standards and were comparable between TAU and SIB, with the exception of a hemorrhagic enteritis vaccine given only to SIB birds. No antibiotics or probiotics were administered at TAU or SIB at any time. Both flocks were monitored over a period of 9 weeks.

Sample collection and processing for *Campylobacter* spp. TAU and SIB birds were monitored for *Campylobacter* at their respective farms at weekly intervals during weeks 1 to 9. Previous analyses of ceca from individual turkeys grown commercially in the same region indicated that, typically, 100% of the birds became colonized by week 3 and, in addition, the different birds tended to harbor the same *Campylobacter* strains (29, 38). Thus, in the current study analysis of *Campylobacter* utilized cecal samples pooled from multiple birds. Each week, 10 birds of each turkey breed were randomly chosen from each farm and euthanized following institutional animal welfare guidelines. The contents of one cecum from each bird were placed into sterile 50-ml conical tubes (Corning Inc., Corning, NY) and thoroughly mixed with a sterile glass rod to produce a composite cecal sample for each farm/breed combination. This led to 36 composite samples, 18 each from TAU and SIB, including nine composite samples of each breed from each farm. For *Campylobacter* isolation, 1 g from the interior of each composite sample was resuspended in 10 ml of Mueller-Hinton broth (MHB; Becton, Dickinson & Co., Sparks, MD). Appropriate dilutions were plated on modified charcoal-cefoperazone-deoxycholate agar (mCCDA; Oxoid Ltd., Hampshire, UK) and incubated microaerobically at 42°C for 48 h as described previously (29). For determination of the number of CFU/g cecal content, colonies with appearance typical of *Campylobacter* were enumerated from the mCCDA plates at the completion of the incubation period.

Characterization of *Campylobacter* spp. Up to six individual colonies from positive samples were selected at random, purified, and preserved at −80°C, as described previously (29). Species designations (*C. jejuni* or *C. coli*) were determined via multiplex PCR as described previously (29). Susceptibility to a panel of antimicrobials, including tetracycline (T), streptomycin (S), erythromycin (E), kanamycin (K), nalidixic acid and ciprofloxacin (Q), and gentamicin (G), was determined as described previously (22). The pansensitive *C. jejuni* strain ATCC 33560 was included in each antimicrobial susceptibility test for quality

assurance, as described previously (22). A subset of isolates representing distinct species and antimicrobial resistance profiles were characterized by multilocus sequence typing (MLST), as previously described (34). A minimum spanning tree (MST) displaying relationships between the MLST sequence types was constructed as previously described (34).

Statistical analysis. Fisher's exact test was used to investigate relative frequencies of strain profiles across age groups, breeds, and farms. Pairwise comparisons of specific strains were also conducted using Fisher's exact tests. All calculations were conducted using the EXACT statement and FISHER option within SAS PROC FREQ (SAS Institute, Cary, NC).

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